

## OXYGEN CONSUMPTION OF TEMPERATURE-ACCLIMATED TOADFISH, *OPSANUS TAU*

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Temperature acclimation in poikilotherms has long been recognized in terms of its physiological consequences: in nature, by the ability of a poikilothermic species to maintain fairly constant activity over a range of habitat temperatures; in the laboratory, by the compensation of metabolism occurring in days or weeks after transfer to a new temperature (Bullock, 1955). The latter has generally been assayed by determinations of oxygen consumption, and a large number of cases have been reported (Bullock, 1955; Prosser, 1962). Among vertebrates, Kanungo and Prosser (1959) showed that acclimation to 10° compared with 30° increased the standard oxygen consumption of goldfish by 25% when measured at 20°, and 75% when measured at 25°. Roberts (1960) found that 5°-acclimated carp consume 60-70% more oxygen at 20° than 20°-acclimated animals. In sunfish, Roberts (1967) has shown that compensation of standard metabolism is almost complete for fish acclimated in the range of about 10° to 20°.

Previous studies in this laboratory have dealt with the role of the protein synthetic system in relation to the biochemical basis of temperature acclimation (Haschenmeyer, 1968; 1969a; 1969b). The toadfish, a marine fish of wide distribution, was used as the experimental animal in preference to other available species because of its adaptability to laboratory conditions and experimental procedures. Although oxygen consumption of toadfish at varying oxygen tensions has been reported (Hall, 1929), no data are available on the effect of temperature acclimation on the metabolism of this species. Therefore, a study has been made of oxygen consumption of toadfish under conditions of acclimation and handling identical to those used in the previous studies.

### MATERIALS AND METHODS

#### *Animals*

Toadfish of intermediate sizes, 200-280 g, were obtained from the Supply Department at the Marine Biological Laboratory, Woods Hole. Control fish were kept in running sea water aquaria at  $21^{\circ} \pm 1^{\circ}$ , the temperature of the sea water supply. Cold-acclimated fish were kept for 9-12 days in similar aquaria maintained at  $10^{\circ} \pm 1^{\circ}$  using the refrigerated sea water supply at the Marine Biological Laboratory. They were starved throughout the acclimation period. Control fish were starved about 7 days. Before measurement of oxygen consumption at  $22^{\circ} \pm 1^{\circ}$ , cold-acclimated fish were transferred to 15° sea water and then to 21° sea water for 1-3 hours, to permit gradual adjustment to the temperature of measurement.

*Oxygen measurement*

A cylindrical lucite vessel, 6 liters volume, with ports for filling, air removal, and introduction of the electrode, was used as the experimental chamber. One end could be removed to introduce the animal and then sealed with a greased rubber gasket. A polarographic oxygen electrode designed by Kanwisher (1959, 1962) was used to continuously monitor the oxygen content of the sea water. Some characteristics of this type of electrode have been reported recently (Carey and Teal, 1965). An external magnet was used to rotate a small stirring bar at the electrode face (Kanwisher, 1959) and to agitate a second stirring bar suspended in a mesh bag which served to mix the contents of the chamber. The signal from the electrode was calibrated at the top of the scale with well aerated sea water at 22° and at the base line with water deaerated with nitrogen gas.

For each measurement the chamber was filled with fresh sea water, the fish was gently introduced, and the chamber sealed excluding air bubbles. The fish quickly settled on the sloping bottom of the chamber and generally did not move from this position throughout the measurement. The decrease in oxygen concentration in the

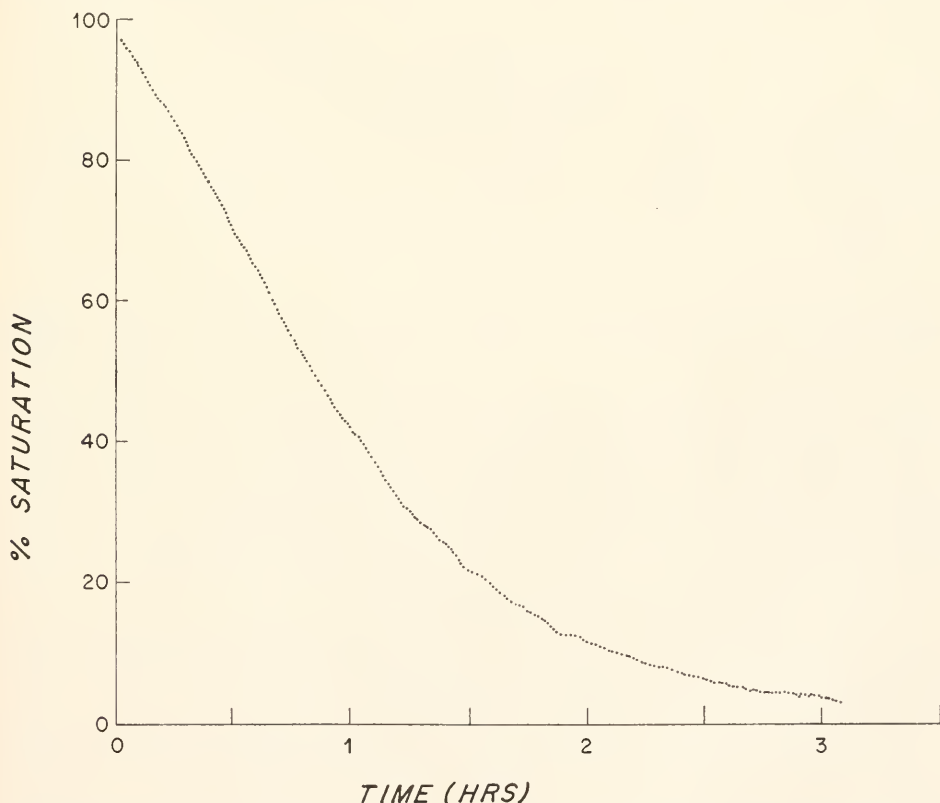


FIGURE 1. Polarographic oxygen electrode recording of oxygen consumption by a toadfish in a closed chamber.

chamber was followed for at least two hours, with oxygen values recorded automatically 75 times per minute.

Calculations of oxygen consumption were based on the linear decrease in per cent oxygen saturation in the range of 40–70% saturation. Because the toadfish is an oxygen conformer (Hall, 1929), it is necessary that all data be obtained in the same range of oxygen tensions. The observed values, ranging from 20% to 40% per hour, were corrected for a background decrease of 3% per hour observed in the absence of an experimental animal and were then converted to ml  $O_2$ /hr using the water volume of the chamber (5.9 l) and a value of 5.09 ml  $O_2$ /l for air-saturated sea water of salinity 32‰ at 22° and one atmosphere total pressure.

## RESULTS

Figure 1 illustrates the results obtained from the continuous measurement of oxygen consumption of a toadfish in a closed respiratory chamber. The utilization of oxygen was essentially linear with time down to about 40% saturation; below this level the decay was exponential. A plot of  $\log O_2$  vs. time was linear in the range of 60% down to 3% saturation, indicating a first-order dependence of oxygen con-

TABLE I  
*Oxygen consumption of control (21°)-toadfish and  
10°-acclimated toadfish at 22°*

Acclimation temperature	Number of animals	Average weight (grams)	ml $O_2$ /hr/kg	% increase
21°	4	264	24.4 ± 3.4 (S.D.)	—
10°	8	243	34.6 ± 6.2	42%

sumption on oxygen concentration. This observation is consistent with Hall's (1929) findings for the toadfish. At the very low oxygen levels reached at the end of some experiments, the fish showed stronger and more rapid opercular movements but appeared normal and unharmed when returned to aerated sea water.

The collected results for oxygen consumption measured at 22° of toadfish acclimated to 10° or to 21° are given in Table I. The cold-acclimated fish show 42% greater oxygen utilization than the control 21° group; analysis by the t-test indicates the difference between the means to be significant at  $P = 0.01$ . The small difference (9%) in average weights of the two groups is not sufficient to account for the observed difference in oxygen consumption. All of the toadfish remained quiet during transfer from one temperature to another and throughout the course of the oxygen measurement. Thus, there was no indication that the higher oxygen consumption of the cold-acclimated group was due to stress or activity. It may, therefore, be attributed to metabolic compensation.

In addition to the measurements at 22°, one fish from each acclimation group was measured in refrigerated sea water at a starting temperature of 12°. The chamber was partially insulated so that the temperature rose only 5° during the two hour period of the experiment. Under these conditions the control fish consumed about 10 ml  $O_2$ /hr/kg; the 10°-acclimated fish consumed about 20 ml

O<sub>2</sub>/hr/kg. Although these results are not complete, they do indicate that a difference between the two acclimation groups can be observed at lower temperatures of measurement as well as at 22°.

### DISCUSSION

Previous studies on the effect of varying environmental temperatures in toadfish have shown: (1) that fish acclimated for one–two weeks at 10° show about 75% greater protein synthetic capacity in liver than control 21° fish when incorporation of radioactive amino acids is measured *in vivo* at 21° (Haschemeyer, 1968); (2) that an aminoacyl transferase functioning in the protein synthetic pathway is increased in cold-acclimated fish (Haschemeyer, 1969a); and (3) that polypeptide chain assembly is significantly faster in cold-acclimated animals (Haschemeyer, 1969b). These changes are not observed in toadfish subjected to only one or two days at 10°. In every case the changes appear to be associated with a compensatory response to the lower temperature, *i.e.*, temperature acclimation.

The present studies were carried out to determine whether toadfish, when assayed for oxygen consumption, would show physiological compensation of metabolism comparable to that observed in other vertebrate fish after cold acclimation and consistent with the molecular changes occurring in the protein synthetic system. As in the previous investigations, acclimation temperatures of 10° and 21° were used. These are within the expected range for capacity adaptation (with maintenance of normal functions), as discussed by Prosser (1967) and Roberts (1967). The results show that at a measurement temperature of 22°, cold-acclimated (10°) toadfish have a significantly greater rate of oxygen consumption (about 42%) than control (21°-acclimated) fish. It therefore appears that toadfish develop at least partial metabolic compensation after 9–12 days at 10°; whether a longer period might produce a more complete compensation, as in sunfish (Roberts, 1967), is not known. The magnitude of the effect falls within the range observed for other poikilothermic vertebrates (Prosser, 1962) and is comparable to the changes observed at the molecular level in the toadfish. Further studies on the mechanism of these changes are in progress.

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### SUMMARY

Oxygen consumption of toadfish was measured in a closed respiratory chamber using a polarographic oxygen electrode. Cold-acclimated (10°) toadfish were found to consume oxygen at a rate 42% greater than control 21°-acclimated fish, when both groups were measured at 22°. The results indicate that toadfish are capable of partial metabolic compensation in response to low temperatures, comparable to that observed in other poikilothermic vertebrates. The overall metabolic effect appears to correlate well with changes observed in the protein synthetic system of toadfish liver under the same acclimation conditions.

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